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AN ELECTRON MICROSCOPIC STUDY ON PEMPHIGUS VULGARIS OF THE MOUTH AND THE SKIN WITH SPECIAL REFERENCE TO THE INTERCELLULAR CEMENT*

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The mechanism and the primary defects which lead to acantholysis in pemphigus vulgaris was regarded by Wilgram *et al.* (1) to consist of the damage to the tonofilaments as the primary event, whereas Braun-Falco and Vogell (2, 3) considered the desmosomes to be the chief site of involvement. The recent finding by immunofluorescent methods that auto-antibodies present in the sera of patients with pemphigus vulgaris are bound to an intercellular substance of stratified squamous epithelium, particularly that of the oral mucous membrane (4), has directed us to this study of the intercellular cementing substance of normal, pre-acantholytic, and acantholytic cells of the oral and cutaneous lesions of pemphigus vulgaris. Oral lesions were studied because their cementing substance is more abundant (Fig. 1a) than that of the skin (Fig. 1b). The fact that the initial lesion of pemphigus vulgaris often occurs in the mouth has also interested us.

MATERIALS AND METHODS

Five oral and seven cutaneous lesions of pemphigus vulgaris were used. Small tissue blocks were immediately fixed in a 1% solution of osmic acid which had been adjusted to pH 8.0 with veronal buffer. After fixation for one and a half hours, they were dehydrated and embedded in Araldite. Thin sections cut on an LKB Ultrotome were stained with 1% uranyl acetate in 50% ethanol and restained with Reynolds' lead citrate solution (5), and were then examined in an RCA EMU-3G electron microscope.

OBSERVATIONS

1. *Oral lesions.* In the vicinity of acantholytic areas desmosomes were found still intact (Fig. 2), while the intercellular cement was largely dissolved (Fig. 2). In early acantholytic areas remnants of the intercellular cementing substance could still be seen in the dilated intercellular spaces as a loosely aggregated mass (Figs. 3a, 3b) or as an amorphous or fuzzy material coating the surface of unlocked villi (Fig. 3a). In some areas masses of serum exudate filled the intercellular spaces (Fig. 4a), but not the space between the basement membrane and the basal cells (Fig. 4b). This exudate dilated the intercellular spaces while the desmosomes were still intact (Fig. 4a), suggesting that not the disruption of desmosomes, but the dissolution of the cement occurs first in the formation of the bullae. A large number of granules varying from 0.3μ to 1.0μ in diameter was seen in the cytoplasm of both acantholytic and preacantholytic cells (Figs. 3a, 3b, 4a). Many of them were aligned near the plasma membrane (Figs. 3a, 3b). In some places these granules were seen merging with the plasma membranes and discharging their contents into the intercellular spaces. They resembled both the membrane-coating granules of Matoltsy and Parakkal (6) because of their peripheral location and the discharge of their contents into the intercellular spaces, and those granules described in our paper on the human posterior buccal mucosa (7) because of their size and the coalescence of smaller granules into larger ones. In contrast to the normal membrane-coating granules which have cristae (6), however, or to the granules found in the posterior buccal mucosa which often reveal vesicular contents (7), these granules in the pemphigus lesions contained amorphously dense material (Figs. 3a, 3b) which appeared somewhat similar to the dissolved intercellular cement (Figs. 3a, 3b).

2. *Cutaneous lesions.* In the skin lesions, desmosomes of preacantholytic cells in the vicinity of blistered areas were also intact (Fig. 5). The desmosomes and attached tonofilaments were fairly well preserved even in cells partially acantholyzed, while the initial

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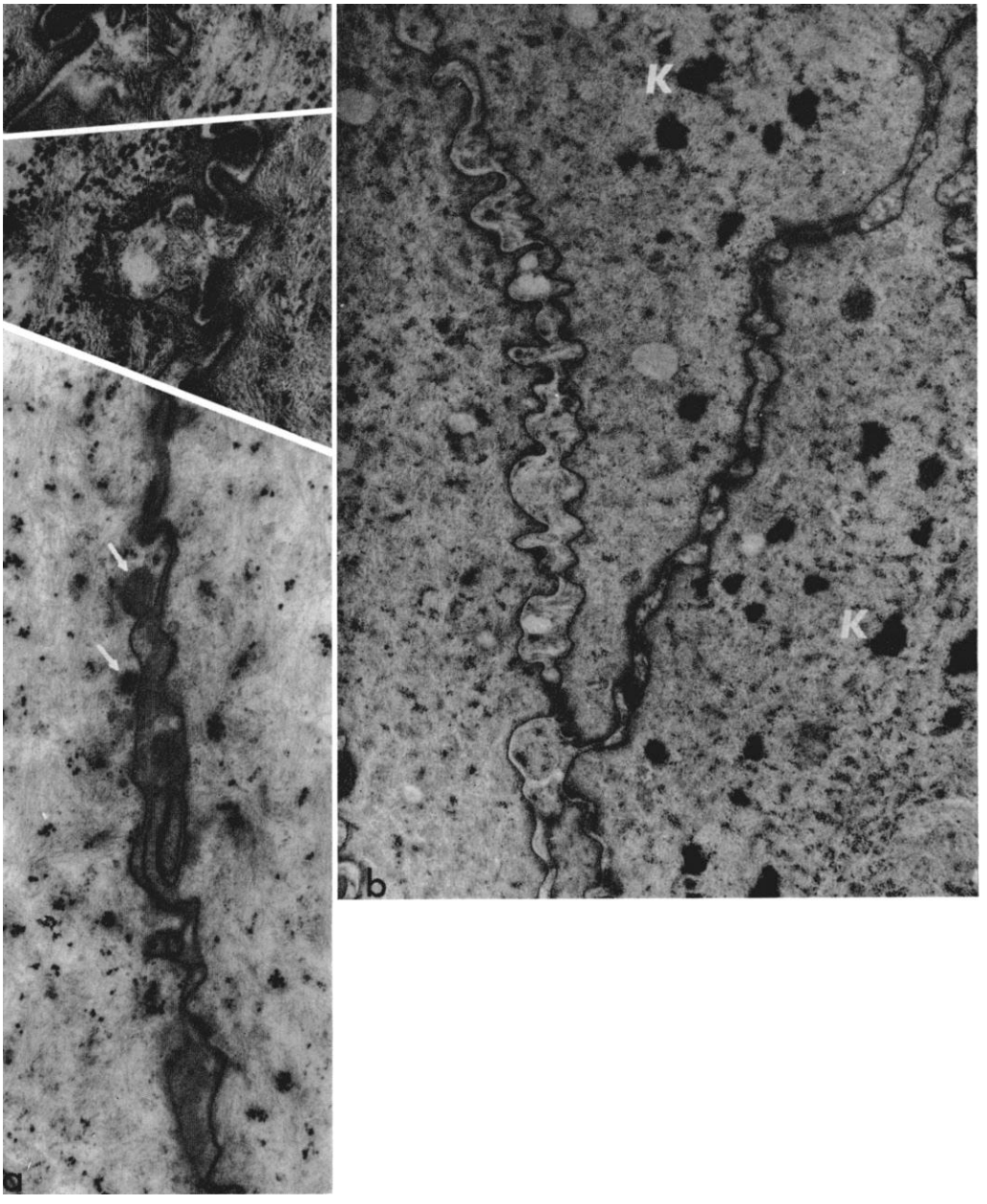

FIG. 1a. *Cementing substance* of the normal human buccal mucosa is composed of admixture of structural elements (two top pictures) and amorphous substance (third picture). Arrows: membrane coating granules. × 44,000, 44,000 + 30,000.

FIG. 1b. *Cementing substance of the upper epidermis* is also made up of structural as well as amorphous substances. K: keratohyaline granules.

change of acantholysis, i.e., the widening of the intercellular spaces and the dissolution of the intercellular cement had already taken place (Fig. 6). The preservation of the desmosome-tonofilament complex in partially acantholyzed cells was typically observed along the upper surfaces of the basal cells from which the overlying squamous cells had recently

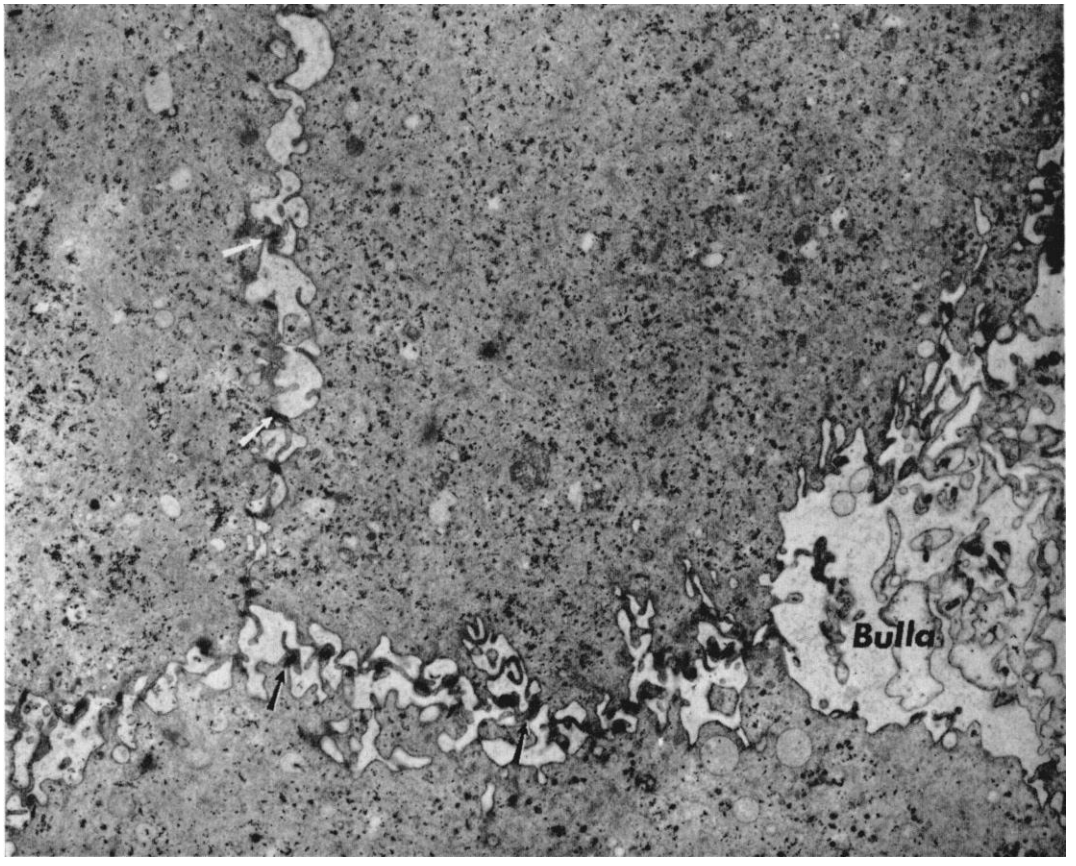

FIG. 2. *Resolution of the intercellular cement* and wide intercellular spaces which represent the very initial stage of acantholytic bulla formation. The desmosomes (arrows) connecting these cells, however, are intact. Oral lesion. × 30,000.

been detached (Fig. 7a and Fig. 7b). On the other hand, in many places where the intercellular cement was preserved, even those cells in which typical retraction of tonofilaments from desmosomal attachment plates and the subsequent disappearance of the desmosomes (as described by Wilgram *et al.* (1)) had occurred, remained attached firmly to their neighboring cells, either healthy or similarly affected (Fig. 8). Therefore, it seemed that whenever acantholysis was about to take place, the cement would dissolve first; but, on the other hand, as long as the cement was preserved, the cells remained attached, regardless of the presence or absence of the tonofilament-desmosome complex. Attachment of the basal cells to the basement membrane was, again, very firm; here, both the cement and the half-desmosomes were well preserved even in severely damaged areas (Figs. 9a, 9b).

DISCUSSION

To the two views which presently exist (1, 2, 3) concerning the location of the primary defect leading to acantholysis in pemphigus vulgaris, we now add a third: the dissolution of the intercellular cement. Braun-Falco and Vogell (2, 3) have also mentioned the importance of the cementing substance and have described the widening of the intercellular spaces as the first change to occur in acantholysis. They place more emphasis, however, on the decrease in the number of and the morphological changes in the desmosomes (2, 3). In the material used in this study, desmosomes appeared normal in preacantholytic cells in

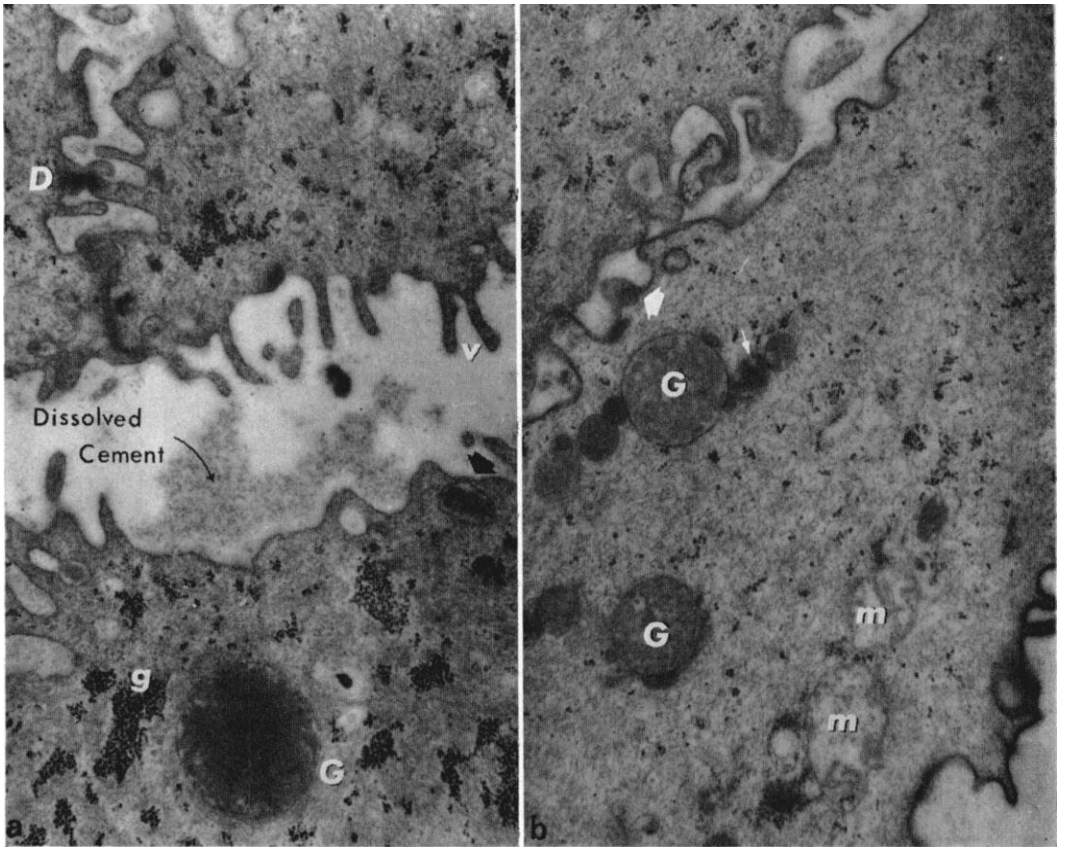

FIG. 3a. *Widened intercellular spaces* contain dissolved cement while one intact desmosome (D) is still present. Unlocked villi (v) are covered with fuzzy filamentous material. There are large granules containing amorphous and moderately dense material (G), one of which is about to discharge its contents into the intercellular space (thick black arrow). The texture of the contents of these granules is somewhat similar to the dissolved cement. g: glycogen particles. Oral lesion. × 30,000.

FIG. 3b. *Large granules (G) are coalescing with smaller ones.* Except for a slight suggestion of vesiculation (thin arrow) in some small granules, their contents are amorphous. One granule is about to discharge its contents into the intercellular space (thick arrow). m: mitochondria. Oral lesion. × 30,000.

the vicinity of blistered areas. They were present with attached bundles of tonofilaments even in partially acantholyzed cells. In the oral epithelium, particularly in the posterior buccal mucosa, tonofilaments and desmosomes are few in number and disappear almost completely in the upper layers. The cellular adhesion, then, appears to depend largely on the intercellular cementing substance which exists in abundance, coating all the surfaces of interlocking pseudovilli (7). Recent findings that the sera of pemphigus patients contain auto-antibodies which bind only with a substance present in the intercellular spaces (4), indicate that in pemphigus vulgaris the intercellular cement is most probably involved. This investigation revealed that the first ultrastructural alteration consists of a damage of the intercellular substance, this observation being made not only in the oral lesions where the intercellular cement is abundant, but also in the skin lesions. In the normal skin intercellular substance is scanty in lower strata, but increases in the upper layers. It consists of various structures and amorphous substances (8). The major portion, however, seems to be derived from the membrane-coating granules of Matoltsy and

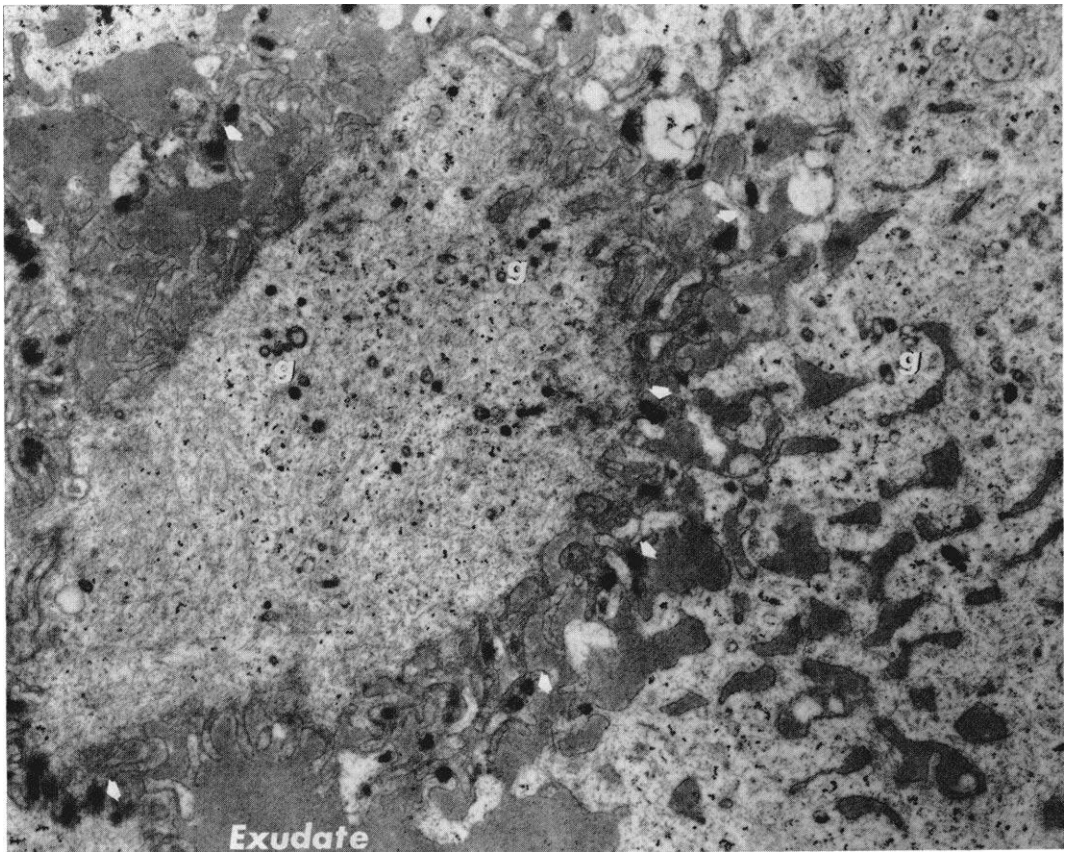

FIG. 4a. *Serum exudate fills the intercellular spaces* and widens them. Desmosomes (thick arrows) are preserved intact. There are numerous small granules (g) similar to the membrane-coating granules. Oral lesion. × 20,000.

Parakkal* (6). In pemphigus vulgaris and particularly in pemphigus foliaceous which involves the cells which normally produce these granules, the membrane-coating granules are not only increased in number, but appear abnormal because of their size (*i.e.*, larger) and internal structure (9). As has been demonstrated in this study, the similar granules which are normally present in the oral epithelium (7) are also deviated from the normal in the oral lesions of pemphigus vulgaris. It is thus possible for these granules to have some pathogenetic significance unless these abnormalities represent secondary changes.

It might be postulated, therefore, that these abnormal membrane-coating granules in pemphigus are either providing cement which is defective in its adhesive capacity or are carrying some substances or enzymes which exert a lytic effect on the intercellular cement; and further, these substances might be able to sensitize the patient to produce the auto-antibodies mentioned above. The speculation that the granules might carry lytic enzymes gains support from our previous observation in which it was found that some granules in the posterior buccal epithelium,

* Although there exists another view regarding the formation of these granules, *i.e.*, they are formed by the infolding of the plasma membranes and thus their function is to take in extracellular substances rather than to discharge cellular products (16), we take the view of Matoltsy and Parakkal (6) because these granules contain a large number of PAS-positive, diastase-resistant neutral mucopolysaccharides which are also present in abundance on the thickened plasma membranes of spinous cells of various tissues (17, 18).

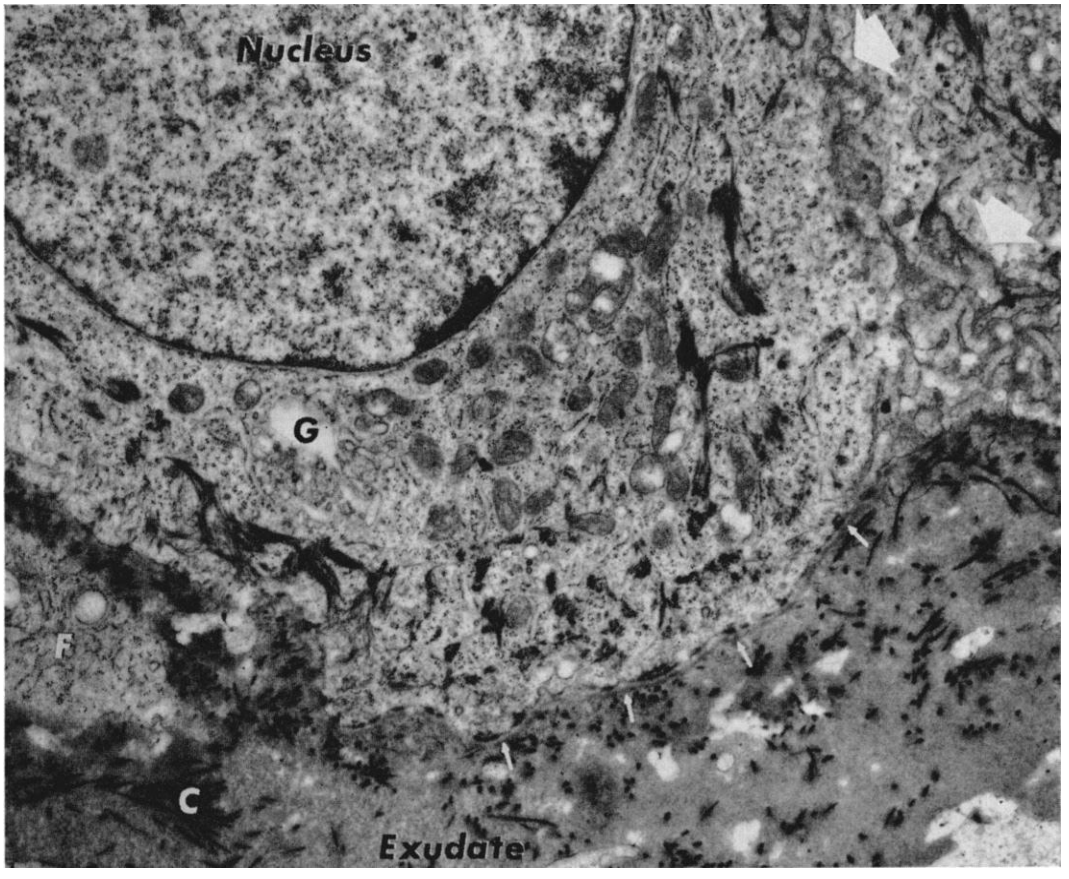

FIG. 4b. *Serum exudate fills the space between two basal cells* (large arrows), but not the space between the basal cells and the basement membrane (thin arrows). C: collagen. F: fibroblast. G: Golgi zone. Oral lesion. × 20,000.

even in the normal condition, contain acid phosphatase, and appear morphologically similar to lysosomes (7). They seem to play an important role in the physiological detachment of the cells in the upper layers. This lytic function has also been attributed to some granules which appear similar to the membrane-coating granules in the upper layers of the normal epidermis by Wilgram (10). In this connection it is particularly interesting to note that in pemphigus foliaceous acantholysis occurs only in the upper layers of the epidermis where the discharge of these abnormal granules takes place.

From the view-point of the auto-antibody reaction against intercellular substance, the distribution pattern of serum exudate filling the intercellular spaces of the oral lesions is particularly interesting because immunofluorescence takes place in exactly the same pattern, and because it is possible for the exudate to carry these auto-antibodies with it. Assuming this to be true then, the negative fluorescence in the basement membrane areas in pemphigus vulgaris (4) could be explained by the absence of serum exudate in the spaces between the basement membrane and the basal cells. The factor which prevents the invasion of the exudate, however, is not easily explainable by ultrastructural differences, since the adhesion of the basal plasma membrane of the basal cells to the basement membrane by the aid of the half-desmosomes is not necessarily tighter than that between the other membranes by the aid of the whole desmosome (11, 12), and liquid nitrogen, for example, can easily separate intact basal cells from the basement membrane (13, 14). Pear-

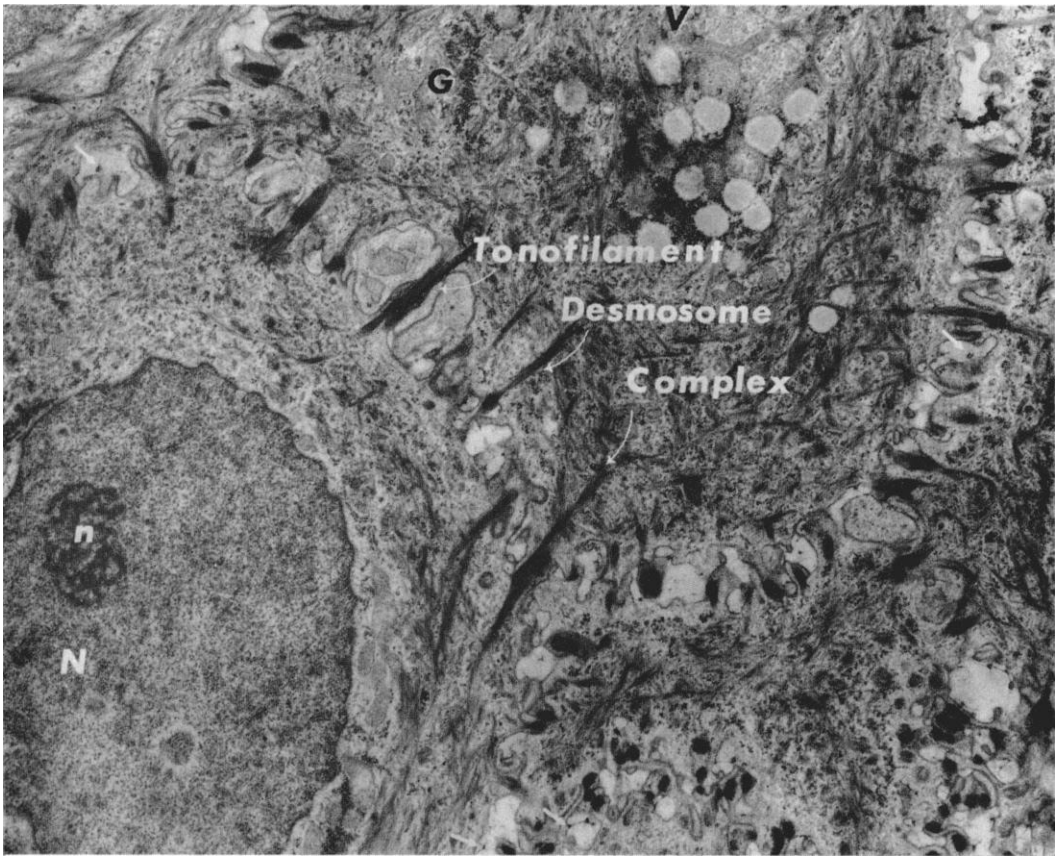

FIG. 5. *Squamous cells in the vicinity of a large acantholytic bulla* show some degenerative changes such as vacuolization of the cytoplasm (V), but the tonofilament-desmosome complex remains normal (curved arrows). Intercellular substance, however, appears to be clumped and stringy in many places (thick arrows). G: glycogen. N: nucleus with some inclusions. n: nucleoli. Skin lesion. × 14,500.

son (14) described a similar separation in epidermolysis bullosa hereditaria letalis. Contrary to our observation and that of Wilgram *et al.* (15), Pearson also described such separation in pemphigus vulgaris (13).

Regarding the retraction of tonofilaments from the attachment plates of desmosomes and the subsequent disappearance of these desmosomes, as Wilgram *et al.* have originally described (1), we have also observed the process repeatedly in old acantholytic cells or in severely damaged cells. Such changes, however, do not necessarily lead to the acantholysis; they can occur in the cells which are still firmly attached to their neighboring cells. The factor which induces such changes might operate independently of that which dissolves the cement and may be non-specific because basically similar changes are also observed in other acantholytic conditions (15).

SUMMARY

1. Several specimens of both oral and cutaneous lesions of pemphigus vulgaris were studied by the electron microscope.

2. Desmosomes appeared to be normal in the vicinity of acantholytic areas, while the intercellular cementing substance was often dissolved. Desmosomes and attached bundles of tonofilaments were also preserved fairly well even in partially acantholyzed cells.

3. On the other hand, whenever the intercellular cement was preserved, the cells in the lesions were kept attached to each other no

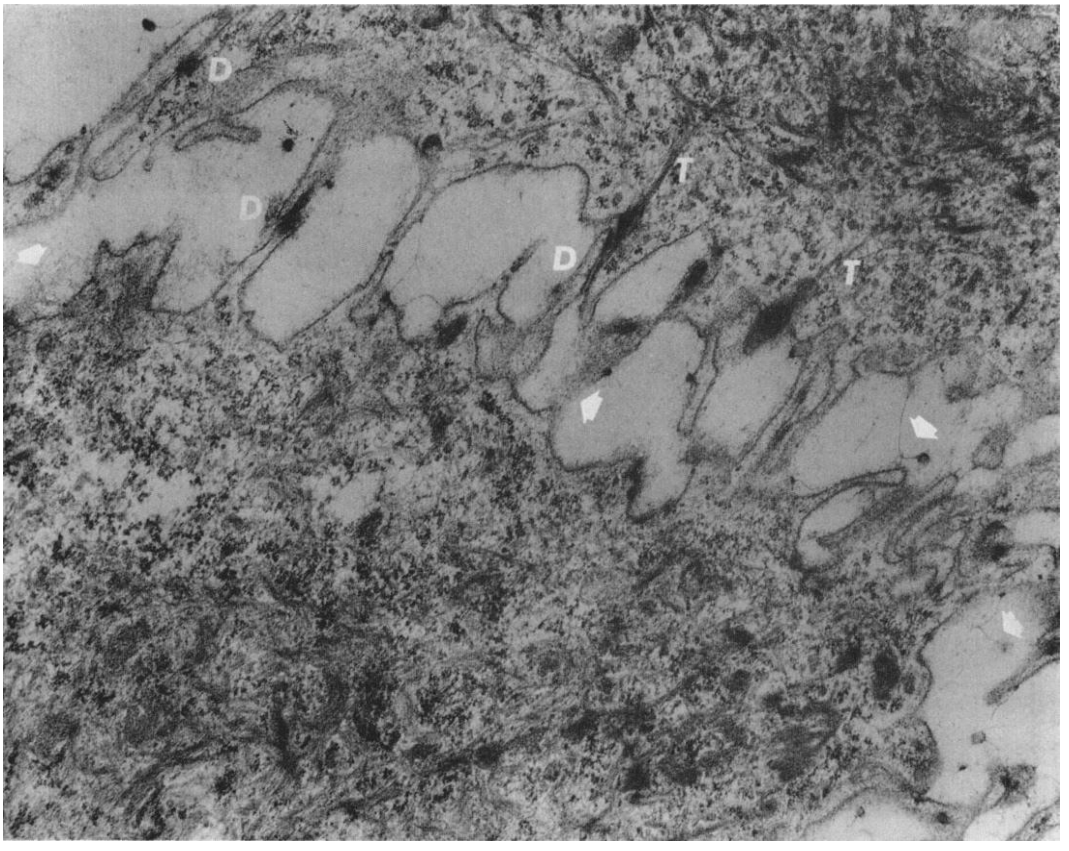

FIG. 6. *Dissolution of the intercellular cement* (arrows) leads to the widening of the intercellular spaces in bulla formation in spite of the persisting desmosomes (D). Dissolved cement appears fuzzy, filamentous or stringy (arrows). T: tonofilament. Skin lesion. × 44,000.

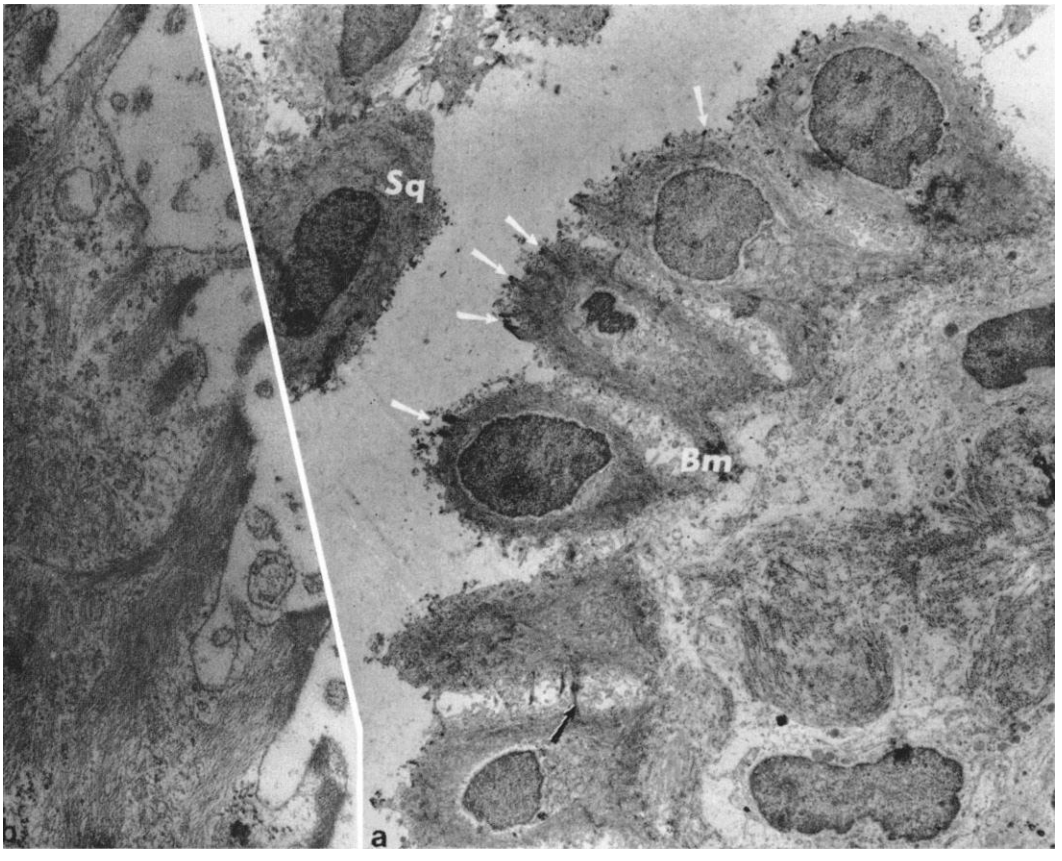

FIG. 7a. *Basal cells lining the floor of a large bulla* are standing on the intact basement membrane (Bm) in firm connection with it. These cells would give rise to the tombstone arrangement of basal cells described by light microscopists. The tonofilament-desmosome complex is intact along the surfaces from which the overlying squamous cells (Sq) have been detached (white arrows). Some desmosomes are present between the basal cells (black arrow). × 4,250.

FIG. 7b. *Enlargement of one of those intact tonofilament-desmosome complexes* shown in Fig. 7a. × 14,500.

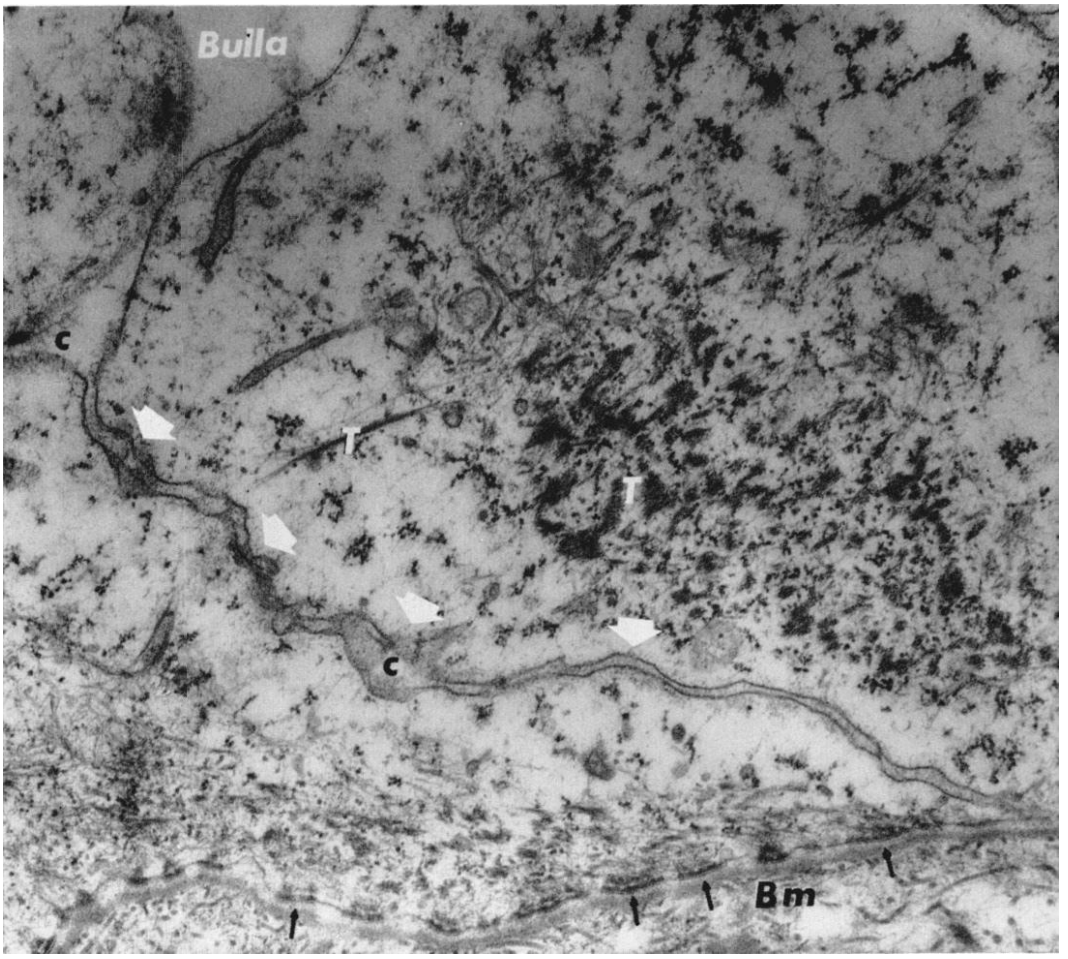

FIG. 8. *Maintained attachment of basal cells* despite the disappearance of desmosomes (thick white arrows) and retraction of attached tonofilaments (T). Intercellular cement (c) is preserved in most areas. Attachment to the basement membrane (Bm) and half-desmosomes (thin black arrows) are intact. Skin lesion. × 30,000.

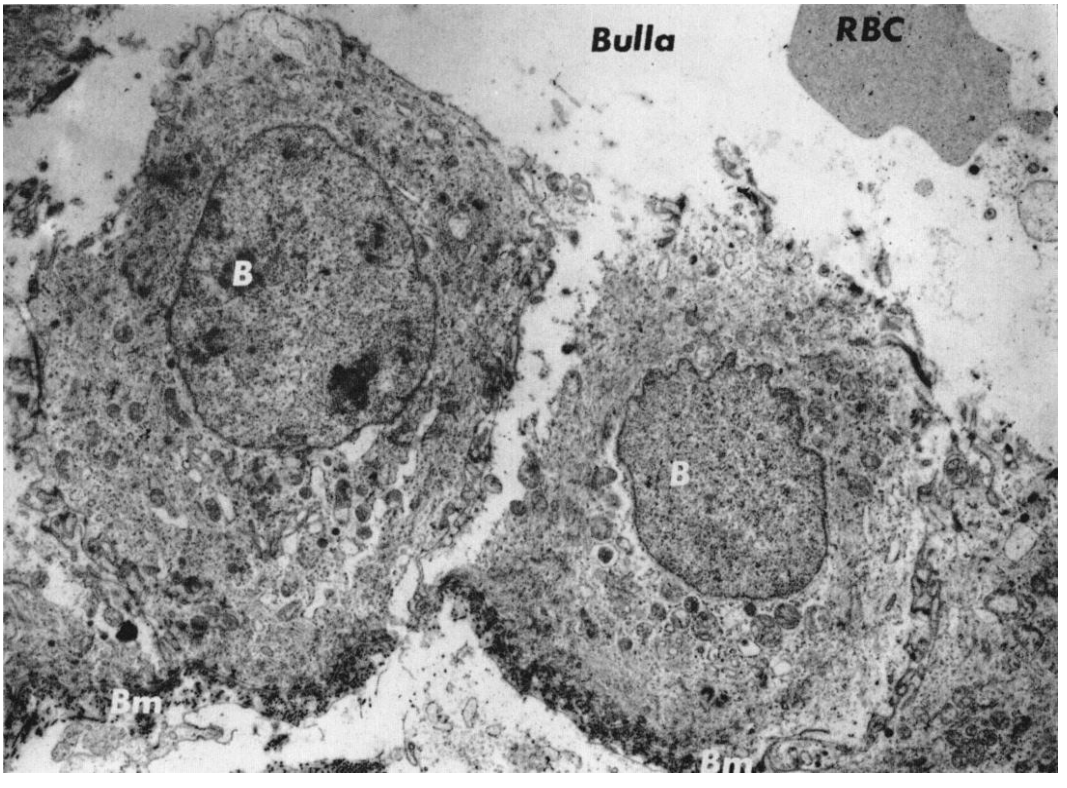

FIG. 9a. *Firm attachment of severely damaged basal cells (B) to the basement membrane (Bm).* Skin lesion. × 5,750.

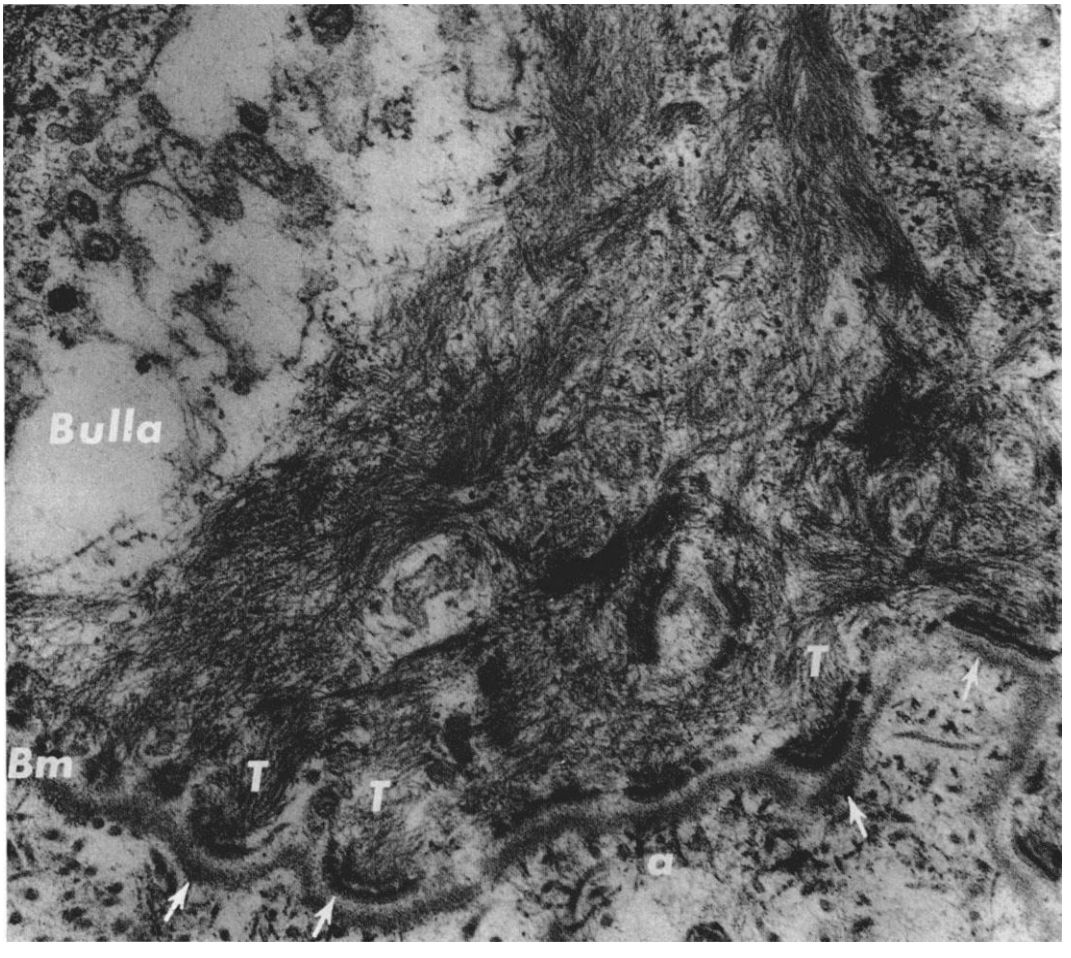

FIG. 9b. *Intact half-desmosome (arrows) and basement membrane (Bm) in severely damaged basal cells.* Attached bundles of tonofilaments (T) are also intact. a: anchoring fibrils. × 44,000.

matter what had happened to the other cellular organelles. Thus cells were kept in firm contact if the cement was preserved, in spite of a complete absence of desmosome-tonofilament complex.

4. In the oral lesions serum exudate often filled the intercellular spaces, but not the space between the basal cells and the basement membrane.

5. From these observations it was concluded that the intercellular cementing substance is first involved in the process which leads to acantholysis. These observations also corroborate the findings of the immunofluorescence study in which the auto-antibodies present in pemphigus patients' sera were shown to be bound only to intercellular substances.

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